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Minireview

Epididymal lithiasis in roosters: In the middle of the way there was a stone

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ABSTRACT

The epididymal region plays an important role in the reproduction of roosters, as it is the site of functions important in the maintenance of fertility, including fluid and calcium reabsorption and sperm surface modifications. About 10 years ago, a reproductive dysfunction characterized by the formation of luminal calcium stones in the epididymal region of roosters was described. This anomaly, known as epididymal lithiasis, is associated with a significant decrease in the fertility of affected roosters. This reproductive anomaly has been observed in multiple countries and is thought to negatively impact the poultry industry; however, the cause of epididymal lithiasis has not been fully determined. Several hypotheses have been proposed to explain the origin of epididymal lithiasis, including the presence of an infectious agent within the epididymal region, an autoimmune response, increased dietary calcium and vitamin D3 intake and the presence of genetic susceptibility factors; however, none of these has been proven to be the primary cause of the calcium stone formation. Nonetheless, considerable evidence suggests that regardless of the primary cause of epididymal lithiasis, this anomaly could result from a hormonal imbalance or a local impairment of calcium homeostasis in the epididymal region. The objectives of this mini-review are to 1) summarize the reproductive alterations observed in animals affected by epididymal lithiasis, 2) discuss the hypotheses proposed to explain the cause of luminal stone formation and 3) provide perspectives for future studies of this reproductive disorder.

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Introduction

Consumption of chicken, which serves as a major source of dietary intake for humans, has climbed worldwide, increasing about 20% in the last ten years (MAPA, 2007; USDA, 2010). As a consequence, an increase in chicken production has been necessary to meet increased demand. Despite this increased demand, chicken production is limited by fertility constraints. Even when kept under controlled conditions, the fertility rate of domestic roosters peaks at 96% at 37 weeks of age. This period of peak fertility is followed by a drastic and rapid decrease in reproductive success, with fertility levels reaching 5% at 110 weeks (Rosenstrauch et al., 1994; Muncher et al., 1995; Weil et al., 1999).

Furthermore, a recent study described a reproductive dysfunction characterized by the presence of calcium stones within the rooster epididymal region that is associated with early loss of fertility in affected

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animals (Janssen et al., 2000). This anomaly, termed epididymal lithiasis, was first identified in the United States and Japan, where its prevalence was about 75%. Epididymal lithiasis was later described in other countries, including Brazil, where lithiasis affects up to 90% of roosters (Mahecha et al., 2002), and Colombia (Personal communication), indicating that the occurrence and distribution of this anomaly may be wider than originally thought.

The avian genital system

In birds, the genital system is composed of the testes, epididymal region, deferent duct and phallus (Fig. 1) (Lake, 1981). The epididymal region is positioned at the dorso-medial surface of the testes and consists of the rete testis, efferent ductules, connecting ducts and a short and non-differentiated epididymal duct (Fig. 1) (King, 1975; Aire, 1979a, 2000). All of these components are surrounded by abundant connective tissue.

Among the components of the epididymal region, the efferent ductules are the most prominent, comprising up to 40%–60% of the epididymal region depending on the species considered (Aire, 1979b; Clulow and Jones, 1988; Oliveira et al., 2007). These ductules can be divided into two continuous segments with distinct morphological characteristics: the proximal and distal efferent ductules. The proximal efferent ductules are characterized by wide lumen and highly folded epithelium, whereas the distal ductules have narrower lumen and fewer epithelial folds (Fig. 1C–D) (Aire, 1979a; Lake, 1981). Both regions are lined by a columnar epithelium formed by non-ciliated and ciliated cells, the latter being more numerous in the distal segments (Aire, 1979a). As in mammals, the efferent ductules of birds are involved in the reabsorption of about 86% of the testicular fluid (Clulow and Jones, 1988); however, they also play an important role in the reabsorption of calcium (Clulow and Jones, 2004). Therefore, the efferent ductules of birds are considered to have an essential function in the concentration and maturation of sperm through their involvement in the maintenance of fluid and calcium homeostasis throughout the avian excurrent ducts.

Epididymal lithiasis

Epididymal lithiasis is a reproductive dysfunction described about 10 years ago (Janssen et al., 2000) that is characterized by the formation of stones within the epididymal region of roosters (Fig. 2A,

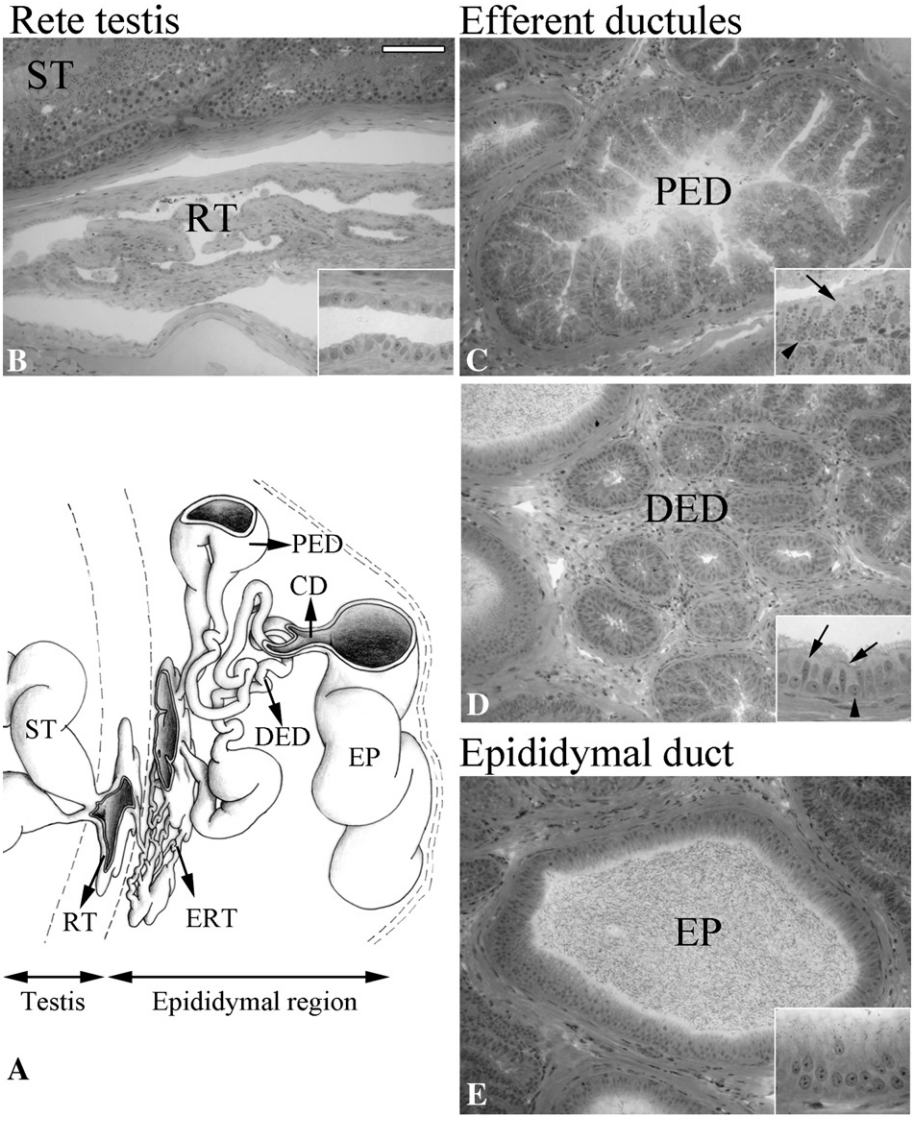

Fig. 1. The avian epididymal region. (A) Schematic representation of the avian epididymal region. (B–E) Histology of the major components of the epididymal region. The inserts highlight the epithelium lining each segment. ST = seminiferous tubules; RT = rete testis; ERT = extratesticular rete testis; PED = proximal efferent ductule; DED = distal efferent ductule; CD = connecting duct; EP = epididymal duct. Arrows in C–D = ciliated cells; arrowheads in C–D = non-ciliated cells. Bar in B–E = 100 μm.

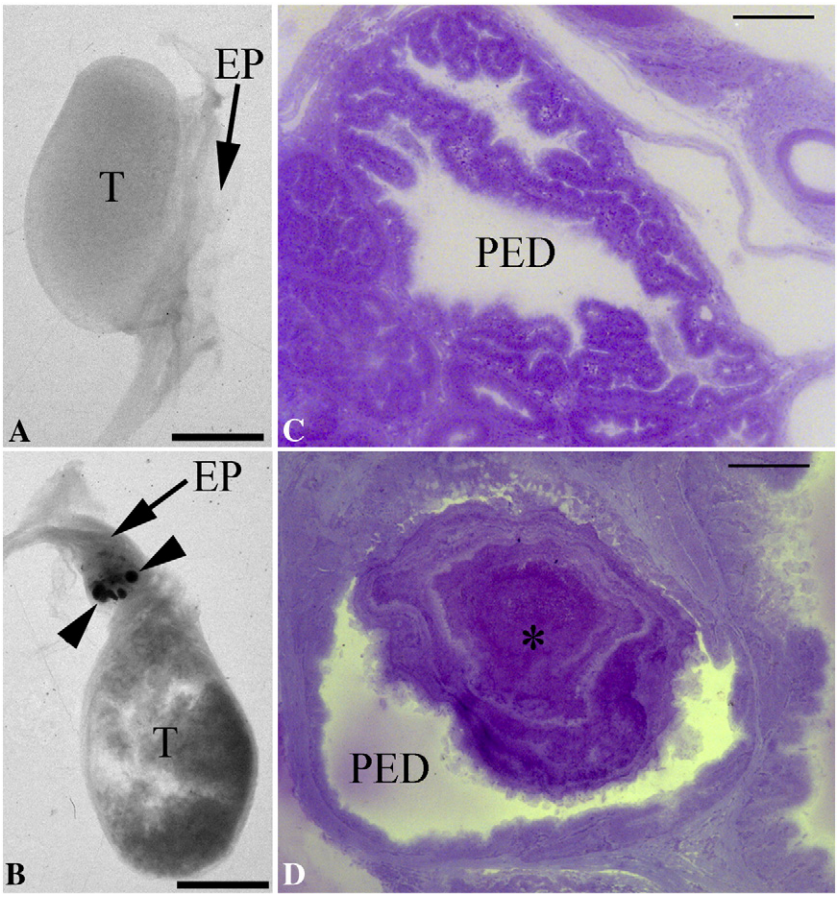

Fig. 2. Epididymal lithiasis. (A–B) Fragments of the epididymal region after clearing in potassium hydroxide and glycerin showing the epididymal region (EP) of non-affected roosters is completely transparent (A), whereas in animals affected by epididymal lithiasis stones (arrowheads) are visible within this region (B). (C) Proximal efferent ductule (PED) of non-affected animal showing a columnar and highly folded epithelium. (D) Proximal efferent ductule (PED) presenting a luminal calcium stone (*). Note the loss of epithelial folding. T = testis. Bar in A and B = 0.5 cm; Bar in C and D = 200 μm.

B). This dysfunction is thought to be restricted to this species, as among the 27 species of domestic and wild birds investigated, stone formation was only found in roosters (Mahecha et al., 2002).

The first signs of epididymal lithiasis are observed by 18 weeks of age, when luminal inclusions become evident within the efferent ductules (Janssen et al., 2000). Between 18 and 26 weeks of age, these inclusions become filled by an opaque fluid, and by 26 weeks, most of these cysts become solid and form stones within the epididymal region. The number and size of stones are thought to increase with age (Janssen et al., 2000). A noteworthy characteristic of affected animals is chronic inflammation characterized by the presence of abundant mononuclear cell infiltrates beginning at 11 weeks of age, several weeks prior to the onset of the appearance of epididymal stones (Janssen et al., 2000). The inflammatory process persists throughout adulthood (Janssen et al., 2000).

Macroscopically, the epididymal stones are irregular in size and shape, with diameters varying from 9 μm to 3000 μm and ranging from smooth or slightly rough to having sharp-pointed surfaces (Janssen et al., 2000; Mahecha et al., 2002). The epididymal stones are composed mainly of calcium (40%–48%), but other substances, including oxygen (28%), carbon (23.5%), sulfur, magnesium, sodium, phosphorus and organic matter are also present (Janssen et al., 2000; Mahecha et al., 2002). Microscopically, the calcium stones are characterized by the presence of a calcified nidus at the core surrounded by alternating layers of calcified and organic matter (Mahecha et al., 2002). It is also common to observe the presence of sperm attached to the surface of the stones and sloughed cells within the stones (Janssen et al., 2000; Mahecha et al., 2002).

Reproductive alterations

All studies to date have shown the efferent ductules to be the segment most affected by epididymal lithiasis (Janssen et al., 2000; Mahecha et al., 2002; Boltz et al., 2004, 2006; Jackson et al., 2006; Oliveira et al., 2008). This observation is based not only on the fact that the calcium stones are formed and retained within the lumen of the ductules, but also on the drastic morphological alterations observed in these ductules (Fig. 2C, D) (Janssen et al., 2000; Mahecha et al., 2002; Boltz et al., 2004, 2006; Jackson et al., 2006; Oliveira et al., 2008). Some efferent ductules present reductions in epithelial folding and height, decreased luminal area and discrete cytoplasmic vacuolization in non-ciliated cells, whereas in other ductules, these vacuoles occupy most of the cytoplasm (Janssen et al., 2000; Mahecha et al., 2002). In addition, numerous mononuclear cell infiltrates are observed adjacent to the efferent ductules (Janssen et al., 2000; Mahecha et al., 2002; Boltz et al., 2004, 2006; Jackson et al., 2006; Oliveira et al., 2008). In some areas, recanalization of these ductules occurs, possibly as an attempt to isolate the luminal stones and allow free transit of testicular secretions and spermatozoa; however, in most affected areas, efferent ductules atrophy is observed (Mahecha et al., 2002). Although the presence of epididymal stones in other segments of the epididymal region is not a frequent finding, stones can occasionally be found in the lumen of the epididymal duct surrounded by mucous substances that may facilitate their passage to the cloaca and excreta (Mahecha et al., 2002).

In contrast to the observation of the aforementioned alterations in the efferent ductules of most affected animals, testicular alterations are generally mild, as no significant changes are found in the volumetric

proportion of the interstitial tissue, seminiferous tubule lumen and epithelium or in the of Sertoli cell population (Oliveira et al., 2008). Furthermore, no differences in sperm production per gram of testis are observed between affected and non-affected roosters (Janssen et al., 2000). In the testes of some affected roosters, however, it is possible to observe areas with moderate sloughing of seminiferous tubules. In some cases, the seminiferous tubules are characterized by a reduced diameter and intense cellular sloughing. In these tubules, the seminiferous epithelium is formed mostly by Sertoli cells and a few spermatogonia, and mononuclear cell infiltrates may also be present (Mahecha et al., 2002). The rarity of major morphological alterations within the testis of roosters affected by epididymal lithiasis reinforces the proposition that the testicular effects of this disorder may be secondary to alterations found in the ducts of the epididymal region.

Affected roosters present a decrease of approximately 65% in circulating levels of testosterone. This decrease in testosterone is paralleled by an increase in the frequency of Leydig cells, which has been interpreted as an attempt to re-establish normal testosterone levels (Janssen et al., 2000; Oliveira et al., 2008). Experiments using natural mating show that together, all of the alterations in the male genital system caused by epididymal lithiasis result in significantly reduced fertility in affected animals, as the production of fertilized eggs is dramatically decreased (62%) in affected animals. Surprisingly, the artificial insemination of chickens with equivalent numbers of spermatozoa obtained from the semen of affected and non-affected animals does not restore the fertility levels of affected roosters, which still present a 41% reduction in the production of fertilized eggs (Janssen et al., 2000). Therefore, the decrease in fertility in roosters with lithiasis is likely due to alterations in the quality of spermatozoa that could reflect functional disruption of the affected epididymal region, especially in the efferent ductules.

What is the primary cause of epididymal lithiasis?

Although epididymal lithiasis results in severe reproductive impairment in affected animals, little is known about its etiology. Several hypotheses have been proposed to explain the cause of epididymal lithiasis. These hypotheses suggest that luminal calcified stones form within the efferent ductules due to (1) a pathological agent, (2) an autoimmune response, (3) dietary vitamin D3 and calcium intake or (4) genetic susceptibility.

Hypothesis 1. A pathological agent.

This hypothesis was proposed as a result of the common observation of abundant mononuclear cell infiltrates within the epididymal region of affected animals (Janssen et al., 2000; Mahecha et al., 2002; Boltz et al., 2006; Jackson et al., 2006). One possible cause for these infiltrates is the presence of a local infectious agent. Avian infectious bronchitis virus (AIBV), which shows a tropism for and replicates in ciliated epithelia such as in the respiratory tract and possibly the efferent ductules, has been suggested as a putative infectious cause of epididymal lithiasis (Dhinakar and Jones, 1997; Jackson et al., 2006; Cavanagh, 2007; Shen et al., 2010). Moreover, AIBV infection has been associated with other pathologies in which calcification of soft tissue is common, such as urolithiasis (Niznik et al., 1985; Brown et al., 1987; Fitz-Coy et al., 1988; Glahn et al., 1989). Based on these considerations, recent studies have focused on the association of AIBV infection with epididymal lithiasis. Systematic monitoring of roosters for circulating anti-AIBV antibodies showed that even roosters with undetectable levels of anti-AIBV antibodies developed epididymal lithiasis (Mahecha et al., 2002). In addition, studies in which animals were vaccinated with live attenuated or killed AIBV failed to prove that the virus or the anti-AIBV antibodies are the cause of the epididymal lithiasis, as the non-vaccinated control roosters also developed epididymal lithiasis (Boltz et al., 2006; Jackson et al., 2006); however, these studies demonstrated that the incidence of epididymal stones was greater in animals exposed to AIBV than in unexposed animals and that the onset of epididymal lithiasis was accelerated in exposed animals (Boltz et al., 2006; Jackson et al., 2006). Together, these data indicate that although AIBV has an influence on the development of epididymal lithiasis, it is not the primary factor involved in its etiology.

Recently, another epitheliotropic virus was associated with the rooster genital tract. The avian metapneumovirus (aMPV) was identified by RT-PCR in testis samples and showed a high prevalence in roosters with epididymal lithiasis (Villarreal et al., 2007). Despite these findings, it is not known whether aMPV plays a role in the origin of epididymal lithiasis; however, several other pathological agents have been associated with soft tissue calcification (Ennever et al., 1981; Cisar et al., 2000; Kosowski et al., 2000; Colpan et al., 2004; Reyes et al., 2009), indicating that the hypothesis of a pathological causes of epididymal lithiasis should not be overlooked.

Hypothesis 2. Autoimmune disease.

Another explanation for the observation of chronic inflammation in the epididymal region of roosters with luminal calcium stones is an autoimmune disease. Among the mammalian reproductive organs, the efferent ductules are the segment most permeable to luminal antigens and are thus the primary site of autoimmune responses (Suzuki and Nagano, 1978; Tung and Alexander, 1980). The autoimmunity hypothesis states that the blood-lumen barrier of the efferent ductules in affected roosters is somehow disrupted or at least leakier than in non-affected animals (Jackson et al., 2006). This leakage may result in the passage of luminal content to the connective tissue and the subsequent stimulation of the interstitial immune cells, including the antigen presenting cells and $CD4^+$ and $CD8^+$ T cells that are abundant in the areas adjacent to the efferent ductules (Yoshimura et al., 2005, 2006). These cells are the putative modulators of a local immune response against luminal antigens that results in the recruitment of other immune cells and in the production of antibodies that are secreted to the lumen. It is reasonable to speculate that these antibodies may promote sperm clumping and consequently the initiation of calcium stone formation as previously proposed (Jackson et al., 2006). Further investigations are necessary to determine the role of immune modulation in the development of epididymal lithiasis.

Hypothesis 3. Dietary vitamin D3 or calcium intake.

Based on the constitution of luminal calcium stones, epididymal lithiasis has been suggested to be related to dietary intake of calcium or vitamin D3 (Janssen et al., 2000; Mahecha et al., 2002), which is a secosteroid hormone well-known for its role in maintaining calcium metabolism (de Matos, 2008); however, no correlation has been found between the number of calcium stones or the weight of the testis/epididymal region and the concentration of calcium ingested (Mahecha et al., 2002). Furthermore, no correlation was observed between vitamin D3 consumption and the occurrence of epididymal lithiasis (Jackson et al., 2006). In support of this finding, some rooster strains do not consume food containing high levels of vitamin D3 but do develop calcium stones within the efferent ductules (Mahecha et al., 2002).

Hypothesis 4. Genetic susceptibility.

Another explanation for the origin of this reproductive disorder is genetic susceptibility. *Gallus gallus* is a species that has been genetically selected for about 3000 years (Etches, 1993), resulting in increased egg production and faster bone formation to shorten the slaughter period. Therefore, it is possible that the selection of animals that mobilize calcium more efficiently has resulted in the selection of roosters that express higher levels of proteins that transport or bind to calcium in different organs, including the efferent ductules, potentially resulting in the formation of luminal calcium stones (Mahecha et al., 2002).

Calcium homeostasis impairment: an alternative hypothesis for the origins of epididymal lithiasis

Although several plausible hypotheses have been proposed to explain the origin of epididymal lithiasis, none of them have proven to fully explain this reproductive anomaly. Nevertheless, considering that calcium is the major component of the stones formed within the lumen of the efferent ductules (Janssen et al., 2000; Mahecha et al., 2002), a segment that in birds is involved in the reabsorption of great amounts of calcium (Clulow and Jones, 2004), it is possible that epididymal lithiasis could result from local impairment of calcium homeostasis (Fig. 3) (Oliveira et al., 2008). As a consequence of this impairment, calcium concentrations would be higher in the lumen of the efferent ductules in affected roosters, forming nucleating centers for calcium aggregation (Oliveira et al., 2008).

Calcium reabsorption across the epithelium

Calcium can be reabsorbed by the epithelium through two distinct pathways: the paracellular route, which involves calcium movement through the intercellular space, and the transepithelial route, in which the ions are transported through the cell's cytoplasm. Transepithelial calcium transport occurs in three general steps, comprising (i) calcium entry through the apical plasma membrane, (ii) calcium translocation to the basolateral membrane and (iii) calcium extrusion to the extracellular environment and bloodstream (Hoenderop et al., 2005). Each of these steps depends on the expression of specific proteins. Calcium enters the cell through specific calcium channels located in the apical membrane, named TRPV5 or TRPV6 (**T**ransient **R**eceptor **P**otential **V**anilloid channel), following an inward electrochemical gradient (Hoenderop et al., 2002). Once inside the cytoplasm, calcium ions bind to calcium binding proteins (CaBP), such as CaBP-D9K or CaBP-D28K, which facilitate calcium diffusion through the cytoplasm to the basolateral membrane of the cell (Hoenderop et al., 2005; Bar, 2009). Finally, the extrusion of cytoplasmic calcium may be mediated by the action of at least two transporters present on the basolateral membrane: the Na^+/Ca^{2+} exchanger (NCX) and the **P**lasma **M**embrane calmodulin-dependent **C**alcium **A**TPase (PMCA) (Bindels et al., 1991; Van Baal et al., 1996; Hoenderop et al., 2005).

A transcriptome analysis of the rat epididymis identified the presence of TRPV6, but not TRPV5 in this tissue (Jelinsky et al., 2007). Further studies also demonstrated expression of calcium binding proteins and PMCA in the rat epididymis (Wilhelm et al., 2008; Hamzeh and Robaire, 2010). In contrast, information about the expression of the proteins involved in the transepithelial calcium transport within the avian male genital tract is still lacking. In the avian efferent ductules, calcium reabsorption occurs against a blood-to-lumen electrochemical gradient, as the luminal calcium concentration in these ductules is approximately 1.4 mM, whereas the circulating calcium concentration is about 2.4 mM (Clulow and Jones, 2004). Therefore, it is likely that the bulk of calcium transport in this segment occurs through the active transepithelial pathway rather than through the passive paracellular route (Fig. 3A) (Hoenderop et al., 2005).

Transepithelial calcium transport and its putative association with epididymal lithiasis

Transepithelial calcium transport is influenced by the hormones that modulate the expression and activity of the proteins involved in this process, including vitamin D3, androgens and estrogens. It is well-known

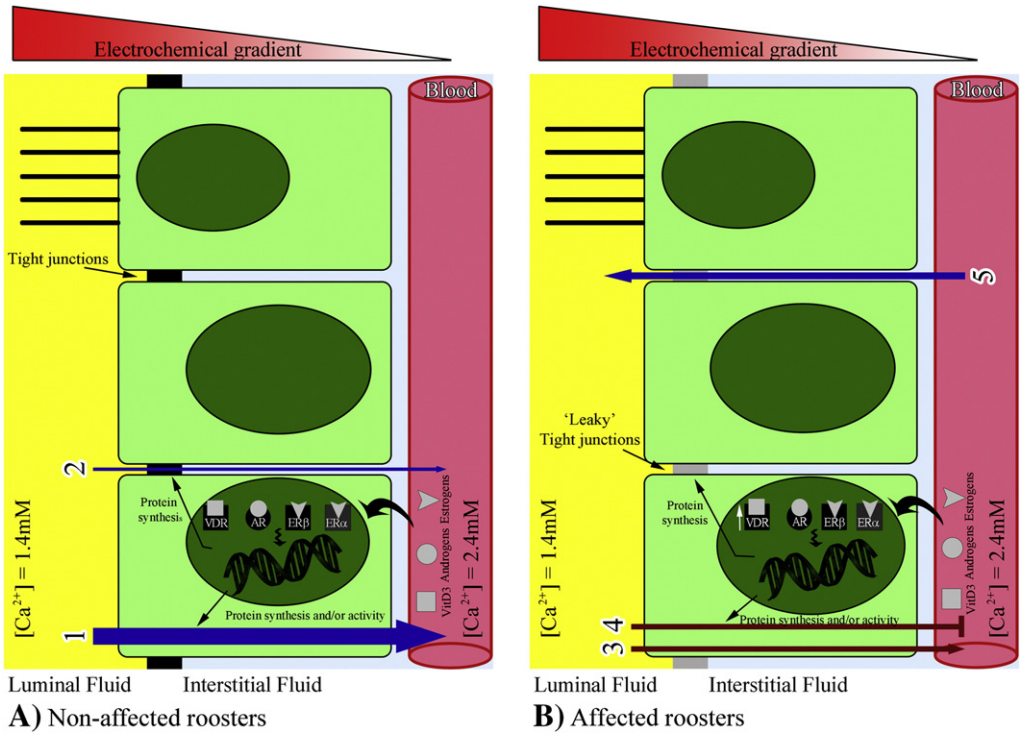

Fig. 3. Diagram showing possible mechanisms for luminal calcium stone formation in the efferent ductules of roosters. (A) In non-affected roosters, calcium is reabsorbed in the efferent ductules against an electrochemical gradient (Clulow and Jones, 2004) through two distinct pathways: the transepithelial pathway (1) and, to a lesser extent, the paracellular pathway (2). These processes are mediated by different proteins (i.e., TRPV5/TRPV6, CaBP-D28K, PMCA and NCX1, junctional complex proteins) and regulated by vitamin D3, estrogens and androgens and their receptors VDR, ERα/ERβ and AR, respectively, which control the synthesis and/or activity of these proteins (Hoenderop et al., 2005; Wada-Hiraike et al., 2006; Fujita et al., 2008; Kong et al., 2008; Braniste et al., 2009; Hsu et al., 2010). (B) In affected roosters, an imbalance in the system responsive to vitamin D3, estrogens and androgens may lead to reduced (3) or impaired (4) transepithelial calcium transport. Alternatively, a hormonal imbalance may result in alterations in tight junction assembly, causing tight junctions to become leaky and calcium to be transported to the lumen following the electrochemical gradient through the paracellular pathway (5). These events may occur individually or synergistically to create a favorable microenvironment for calcium aggregation into the efferent ductule lumen.

that vitamin D3 is one of the most important hormones involved in calcium reabsorption through the transepithelial pathway, as the expression of TRPV5, TRPV6, CaBP-D28K and PMCA are highly dependent on this hormone and its receptor VDR (Cai et al., 1993; Hoenderop et al., 2001, 2002, 2005; Meyer et al., 2007; Suzuki et al., 2008). Androgens and their nuclear receptor AR also influence calcium transport, as high levels of testosterone decrease the expression of TRPV5, CaBP-D28K and PMCA, whereas the opposite effect is observed when testosterone levels are low (Hsu et al., 2010).

Based on these facts and considering that VDR, AR and their ligands directly modulate transepithelial calcium transport, our laboratory investigated whether these receptors are expressed in the epididymal region of roosters and examined their distribution in this segment (Dornas et al., 2007, 2008). Higher levels of VDR were found in the efferent ductules, whereas AR was more highly expressed within the epididymal duct epithelium, suggesting that different segments of the epididymal region may be differentially regulated by vitamin D3 and androgens (Dornas et al., 2007, 2008). Roosters with epididymal lithiasis expressed significantly higher levels of VDR in the epithelium of the efferent ductules than did non-affected animals. In contrast, AR expression levels were not altered in this segment. These data suggest that vitamin D3/VDR signaling can be a key factor in the origin or development of epididymal lithiasis by diminishing or preventing the calcium reabsorption process (Fig. 3B) (Oliveira et al., 2008). On the other hand, nothing is known about the levels of the ligands of these receptors in tissues or circulation. Therefore, it is necessary to investigate the levels of vitamin D3 and androgens in roosters affected by epididymal lithiasis to better understand the physiological significance of the expression patterns of VDR and AR.

In addition to vitamin D3 and androgens, transepithelial calcium transport is also influenced by estrogens. Previous studies have shown that 17β-estradiol induces the expression of TRPV5, TRPV6 and CaBP-D28K proteins and increases the synthesis and activity of PMCA (Van Abel et al., 2002; Dick et al., 2003; Oz et al., 2007). It is well-known that the efferent ductules are the main target for estrogen action among the components of the male genital system, and that estrogen receptors ERα and ERβ are highly expressed within this male genital tract segment including in birds (Hess et al., 1997; Kwon et al., 1997; Oliveira et al., 2010). Thus, considering the high expression levels of ERα and ERβ in the efferent ductules of roosters (Oliveira et al., 2010), which are the site of formation of calcium stones, together with the participation of estrogens in the maintenance of local calcium homeostasis, it is important to address the expression of ERα and ERβ in the epididymal region of roosters with epididymal lithiasis in order to better understand the mechanism of calcium stone formation.

Paracellular calcium transport and its putative association with epididymal lithiasis

Calcium transport may also occur to a lesser extent through the paracellular pathway. If we consider only the paracellular pathway, calcium would preferentially be transported towards the efferent ductule lumen, following the blood-to-lumen electrochemical gradient, a process that — at least under normal conditions — would be avoided or finely regulated by the tight junctions present between the efferent ductule epithelial cells (Ozegbe et al., 2006), similar to the regulation found in mammalian tissues (Hoenderop et al., 2005). If the epithelial junctional complexes in efferent ductules affected by lithiasis became leaky, they would serve as a passage for calcium to the lumen, increasing the luminal concentration of this ion and consequently creating a microenvironment favorable to calcification (Fig. 3B). The presence of leakier junctional complexes in the efferent ductules of roosters affected by epididymal lithiasis has already been proposed as the source of luminal/mucosal substance exchange (Jackson et al., 2006).

The expression of proteins responsible for the assembly of the junctional complexes, including key proteins required for the maintenance of tight junctions, adherens junctions and desmosomes, is highly dependent on hormonal regulation. Not surprisingly, vitamin D3, estrogens and androgens and their respective receptors are important factors in this regulation. Vitamin D3/VDR signaling has been shown to alter the expression profiles of ZO-1, E-cadherin and claudins (Cldn), as Cldn2 and Cldn12, but not Cldn7 and Cldn15 (Fujita et al., 2008; Kong et al., 2008). Furthermore, VDR knockout mice present decreased expression of Cldn1, Cldn5, E-cadherin and ZO-1 and increased expression of Cldn3 (Fujita et al., 2008; Kong et al., 2008). Together, these alterations might explain the increased susceptibility of VDR knockout mice to mucosal injury (Kong et al., 2008) and the induction of paracellular calcium transport as seen in different intestinal segments (Fujita et al., 2008).

In addition, estrogens and androgens also play a role in the modulation of junctional complex assembly. Estrogens modulate the expression of occludins, α-catenin, plectin, α-spectrin and β-spectrin. Both the decrease in estrogen levels and the disruption in estrogen/ERβ signaling in βERKO knockout mice result in the impairment of cell–cell adhesion and the consequent increase in paracellular permeability (Wada-Hiraike et al., 2006; Braniste et al., 2009). Androgen levels are also related to the modulation of testicular Cldn proteins, as Cldn1 decreases after puberty, while Cldn11 increases after puberty, paralleling the increase in testosterone levels (Park et al., 2010).

Considering the complex dependence of junctional adhesion complexes on different hormones and the altered VDR and AR expression patterns within the epididymal region of roosters with epididymal lithiasis, one cannot rule out the possibility that paracellular calcium reabsorption is altered in these animals and contributes to the formation of luminal calcium stones.

Concluding remarks and future perspectives

Studies of epididymal lithiasis have revealed that this reproductive dysfunction is definitely more complex than originally thought. Currently, none of the hypotheses presented can fully explain the development of epididymal lithiasis. Each of the proposed mechanisms may contribute to the formation of luminal calcium stones, but a synergistic action cannot be ruled out. Future studies proposing to further investigate hormonal imbalances in affected roosters to address the roles of the epididymal alterations described to date, including mononuclear cell infiltration and altered VDR and AR expression patterns, and to assess the estrogen responsive system and calcium reabsorption in the efferent ductules of roosters are critical to understand this intriguing reproductive anomaly.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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